Atkinson (J.E.)

With the Compliments of the Author.

THE BOTANICAL RELATIONS OF TRICHOPHYTON TONSURANS.

BY

I. EDMONDSON ATKINSON, M. D.,

OF BALTIMORE.

[*REPRINTED FROM THE NEW YORK MEDICAL JOURNAL, DEC.*, 1878.]

NEW YORK:
D. APPLETON & COMPANY,
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1878.

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The results obtained by those who have made the life histories of the fungous growths, which are the exciting causes of certain diseases of the skin of man and some other mammals, the subject of study, have differed so widely that one is impelled to adopt one of two conclusions: either there exists in these forms of vegetable life a polymorphism exceeding the most extravagant claims of Hallier, or else the methods of investigation adopted by these observers have been sadly inaccurate and exposed to all sorts of adventitious influences. With the best mycologists of the day the opinion prevails that, while a limited polymorphism may be admitted, one may reject, without hesitation, those theories which would embrace in one genetic series the different fungi to which the diseases under consideration have been attributed. On the other hand, a moment's reflection must make it evident that the methods of cultivation employed have been open to the gravest objections, and the cultivations themselves exposed to the most varied contaminations. Results obtained from cul-

[1] Read before the American Dermatological Association, at Saratoga, August 28, 1878.

tivation upon such nutritive soils as sliced vegetables, potatoes, carrots, apples, and the like, must prove absolutely untrustworthy, since it is a matter of every-day experience that all such preparations will inevitably become the hosts of multitudes of spores, which rapidly invade, conceal, and overwhelm the less vigorous spores planted upon them. No accurate worker would, to-day, dream of obtaining truthful results through such unscientific methods.

A much more reliable process than the foregoing has been used by certain mycologists, who claim a high degree of purity for the cultivations conducted by it. According to Brefeld, this method consists in sowing a single spore in a drop of a given nutritive fluid, upon a glass slide resting upon a metallic plate, under a bell-glass, and protected from external influences by having the latter resting in water. ("Botanische Untersuchungen über Schimmelpilze," Heft 1, p. 5). Minute precautions should be used to thoroughly purify all the materials employed. This plan offers manifest advantages over the previous one, but the liability to contamination still remains excessive; for, not only is it impossible to secure the necessary purity of the atmosphere of the bell-glass, but the frequent removals of the latter for purposes of inspection and of supplying the losses of nutrient fluid by evaporation must inevitably lead to the lodgment of adventitious spores of yeast, mould and bacteria, and the like, circumstances, when not fatally interfering with the growth of the special fungus, affording great danger of confounding the latter with intruders of widely different nature.

Desiring to make some study of these parasitic fungi, and being conscious of the imperfections of the foregoing methods, I fortunately made known my difficulties to Prof. H. Newell Martin (in whose laboratory, at the Johns Hopkins University, the more important of my researches have been followed), who suggested to me the method employed by MM. Van Tieghem and Le Monnier in their observations upon the Mucorini, published in the 17th volume of the "Annales des Sciences Naturelles" (pp. 261–399). His plan may be called the cell culture, and, briefly described, is as follows: The cell is constructed by fastening with Canada balsam, upon a glass

slide, a glass ring from four to five millimetres in height, and about fifteen millimetres in diameter. It should be ground flat upon its edges. A thin cover-glass, as thin as can be procured, of the diameter of the ring, forms the roof of the cell. When it is to be used, a drop of nutritive fluid is placed upon the cover-glass, and into this drop the fungus is sown. The cover-glass is then placed upon the ring, with the drop upon its under-surface, a drop of boiled distilled water having been previously placed in the bottom of the cell, to secure the proper atmospheric moisture. The cover-glass is kept in position and protected from the external air by a few minute drops of oil. In pursuing this method it is, of course, necessary to observe all possible precautions to prevent the introduction of foreign spores. The nutritive fluid, the distilled water, and the oil, should be boiled in test-tubes, stoppered with cotton wool and only opened at the instant of using. I have adopted the plan of drawing these fluids into fine pipettes previously subjected to an extreme heat. In this way a drop of the required minuteness can be obtained quite uncontaminated. The cell and cover-glass must be scrupulously clean and all accessory apparatus thoroughly purified. When finished, the cells should be placed side by side in a box, half filled with moist sand, and protected by a lid or a piece of glass. In the winter it will be advisable to keep the box in a water bath at a temperature of from 20° C. to 30° C., or upon a mantelpiece over a fire. In summer no such precautions are necessary.

The cell may now be examined under the microscope, and every part of the drop observed. With thin cover-glasses quite powerful objectives can be used. In my investigations a Zeiss's D objective was most conveniently employed, although it was possible to use with profit the F objective of the same maker. The advantage of the greater amplification of the latter objective, however, was more than counterbalanced by the danger of breaking the cover-glass in obeying the almost irresistible impulse to peer as deeply as possible into the cell. One of Grunow's $\frac{1}{8}''$ objectives of 110° angular aperture was also easily used. With the D objective and No. 4 eyepiece, an amplification of 400 diameters was attained.

I have employed as nutrient fluids Pasteur's fluid, with and without sugar, distilled water, orange-juice, decoction of horse-dung, aqueous humor, gelatine, currant-jelly, and meat infusion. Of these, orange-juice has seemed the most suitable, although I have succeeded with Pasteur's fluids. The horse-dung decoction, so highly recommended by Brefeld, I have found so extravagantly disposed to the development of bacteria that it has been useless in my observations. The acidulated solutions were preferable, on account of their freedom from bacteria.

It must at once be admitted that it is impossible for strange germs to find their way into a cell after its completion under the above-mentioned precautions, unless it be by thrusting their hyphæ between the cover-glass and cell, a proceeding that can easily be detected. It remains to be seen to what extent the culture can be kept pure during the moments occupied in the preparation of the cell. It shall be my endeavor to show this later.

The fungus I selected for cultivation was "Trichophyton tonsurans," taken at different times from the heads of two light-haired boys. After thoroughly washing the affected surfaces, I extracted very short stumps of hairs, with as much of the bulb, or lower part of the shaft, as possible, this being a procedure of much difficulty, since in a large majority of cases the hairs break off outside of the follicular orifices. I selected portions of hairs rather than single spores, partly because it has been with me the rarest occurrence to see a spore sprouting apart from its habitat, the hair; partly on account of the infinitely slender chances of selecting a spore capable of budding in cell cultivation; but chiefly, because the style of germination in a successful cultivation has been so distinctively characteristic, that I have considered the results obtained sufficiently convincing.

It must not be supposed, however, that germination occurs readily in these cells. On the contrary, probably on account of the restricted air-supply, it is, by far, the usual experience to find the cell remain absolutely quiescent, the homogeneous and apparently perfect spores remaining for weeks unchanged, finally to slowly disintegrate. As Van

Tieghem and Le Monnier have remarked, the causes of failure in cell cultures are very different, and by no means obvious. This much is certain: that a large proportion of cells, with hairs full of spores in apparently perfect condition and remaining entirely free from adventitious growth, and kept under observation for many days, show not the smallest sign of development. This indisposition to germinate is not common to all fungous forms when sown in cells, as is proved by the facility with which penicillium and aspergillus shoot out their vigorous hyphæ, when their spores have accidentally or designedly been introduced; and especially by the success with which MM. Van Tieghem and Le Monnier cultivated the various forms of mucor, obtaining even the sexual reproductive process, a development that Brefeld has never observed in fluid cultivations.

Where, however, a successful cultivation is secured, never does a single nor even do a few hyphæ appear; but there is invariably a multitudinous and simultaneous outburst of growth of hundreds of spores, indicating that the conditions of life and development depend upon some special appropriateness of the cell and of the fungus.

The history of a successful cultivation, then, is as follows:

A short, broken hair, extracted with as much as possible of the part within the follicle, containing the more active spores, is secured, and with all practicable dispatch is sown in the nutrient fluid, and the cell completed by laying on the cover-glass. In from twenty-four to thirty-six hours, more usually the latter period, but frequently only after several days, signs of vigorous growth will become evident. The spore mass will be seen to exceed its boundaries of the day before, projecting in a single or double rows or more beyond the hair, both in the direction of its axis and laterally. These spores, whose nutriment has been abundantly supplied, will sometimes be observed to swell to many times their original proportions, attaining sometimes a diameter of .0222 millimetre. (*See* Fig. 2, *b*.) This process is not often observed in its fullest degree, and ceases as soon as hyphæ begin to be freely thrown out. I have been unable to decide whether the insignificant increase in the area occupied by the spore mass is due

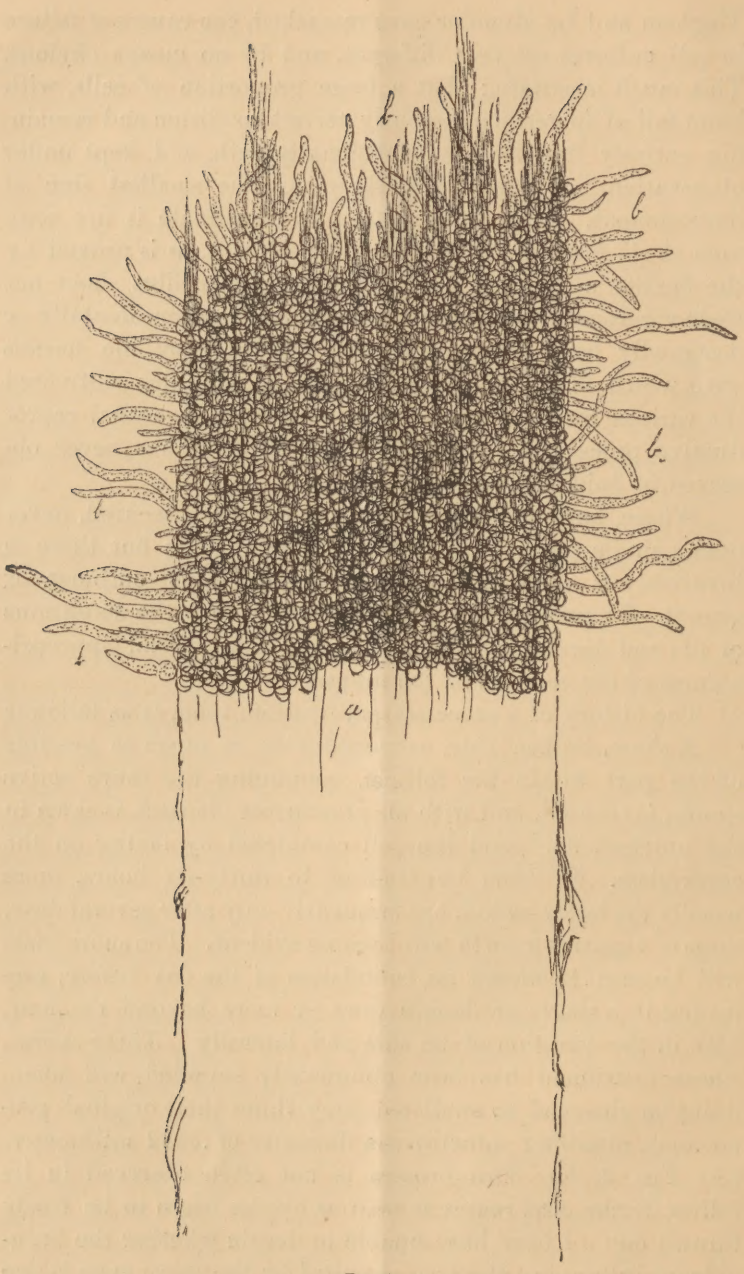

Fig. 1.

to this swelling alone, or to an actual increase in the number of spores by a simple budding, torula-like process, as well. I have not detected the latter in cultivation, but am satisfied that it occurs, since in the growth of the fungus in the hair, just after detachment, many partially budded spores are visible, but their growth seems to be arrested by their transference to the culture fluid. Spores which have swollen to the larger diameter undergo no further development, or throw out short hyphæ, which remain unbranched and whose growth soon becomes arrested. Almost simultaneously with this swelling of particular spores, or even, perhaps, without this having occurred, hyphæ may be seen shooting out from the hair-shaft in hundreds (Fig. 1, *b*), the spores from which they spring, as well as those which have undergone no change, having a diameter varying from .002 millimetre to .005 millimetre for the globular ones; and for the oval or oblong ones from .004 millimetre to .0045 millimetre in breadth, and from .007 millimetre to .01 in length. The hyphæ have at the same time an average diameter of .0025 millimetre or more, and grow, as yet, without dividing and without forming septa. They spring, medusa-like, from the hair, and may occasionally be traced to their proper spores, which may begin to be slightly vacuolated.

The abundance of nutriment being favorable to the formation of a mycelium, the hyphæ now freely branch, and by the third day many have become septate, the segments becoming frequently irregularly bulbous or forming globular swellings (*see* Fig. 2) of very much increased size, .015 of a millimetre or more in diameter. These conditions may be observed exhibited in Fig. 2. By this time, the mycelium begins to form a network of greater or less density, and already at numerous points, both lateral and terminal, short hyphæ have been thrown out, bearing at their terminations globose bodies with granular contents, occasionally vacuolated, and which quickly become separated from the hyphæ by partitions directly transverse to the hyphæ and presenting sporangial characters (*see* Fig. 2, *d*). Most of these sporangium-bearing hyphæ are devoid of septa until the formation of the one representing the columella, but the mycelium from which they arise possesses septa at tolerably

wide intervals (*see* Fig. 2). By about the fifth day the hyphæ and mycelium become freely vacuolated and the sporangia begin to exhibit little aggregations of protoplasm, the future

Fig. 2.

spores. The septa between them, however, and the hyphæ remain quite flat. These sporangia may attain an extreme

diameter of .018 millimetre. Here and there the hyphæ show a tendency to break up into small segments (*see* Fig. 3, B), a process, however, which I have never seen carried to the extremity of spore or brood-cell formation. Occasionally, also, instead of retaining their sporangial characters, the sporangia, as if diverted from their original purpose, develop buds at one or more points of their surfaces, which in their turn become hyphæ. (*See* Figs. 2 and 3, *e*.)

With abundance of nutriment, the tendency of the hyphæ to grow into a closely meshed mycelium is very marked, and but comparatively few distinctive reproductive organs are developed. It is in the underfed cultivations, in those where the actively growing hyphæ soon exhaust the fluid surrounding them and where the remaining vigor is diverted to the formation of reproductive organs, that the growth is followed with most facility and most decided results. In such a cell, while the swelling of the spores is less noticeable, the hyphæ are thrown out precisely as when more abundantly nourished, and for a limited period (thirty-six to forty-eight hours) pursue a similar course. Beyond this point they become less vigorous, their branching becomes arrested, and their advancing growth ceases. Septa usually, though not always, appear in the hyphæ, which sometimes shows bulbous irregularities, as if about to form jointed spores. About the third day some of the hyphæ show sporangium-like enlargements (Fig. 3, *d*), both terminal and lateral, in which vacuoles appear, and which exhibit no columellæ or partitions from the hyphæ, in which by this time vacuoles have become abundant. In instances most favorable to observation, the hyphæ spring directly into the formation of sporangia without a single branching and quite often without the formation of septa, and after very insignificant growth, just as the sexually produced zygospores habitually do. From this point their growth is somewhat indefinite and is evidently controlled by conditions of nutrition. A very few sporangia (Fig. 3, *g*) develop columellæ, but are apt to form no spores; others, with or without septa, form a few diminutive spores; others again, after forming sporangial enlargements, may develop buds which either grow as ordinary hyphæ or swell into sporangial forms, like beads strung to-

gether (Fig. 3, *e*, *f*; Fig. 2, *e*). In the very few instances where this reproductive process reaches completion, the wall of

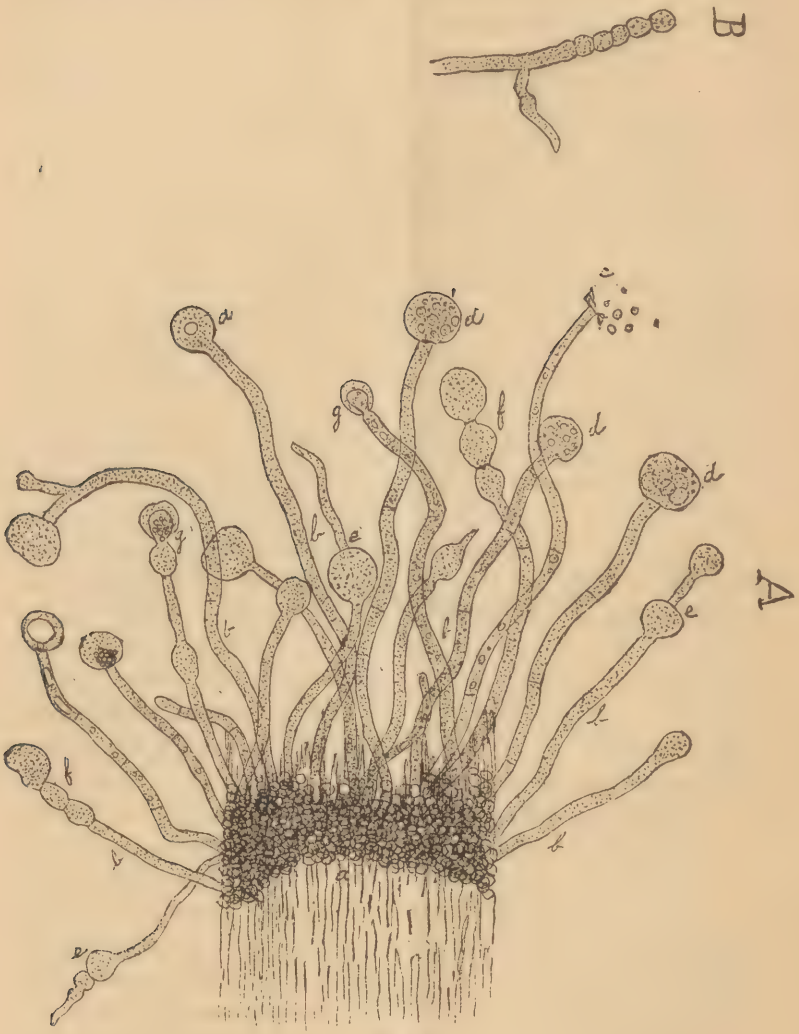

FIG. 3.

the sporangium bursts and four or five minute spores are released (Fig. 3, *c*). Here all signs of development are at an end, and the cell remains unchanged for an indefinite period.

The sporangia show by their varying forms that they have not acquired their full and perfect maturity, and that their development is controlled by the circumstances of their position, so that they betray a singular indecision, so to speak, in their final course.

My experience has not enabled me to speak positively concerning the formation of chlamydo-spores, or spores developed within the hyphæ, as described by Van Tieghem and Le Monnier, in their account of the mucors, nor have I detected any disposition toward the sexual union of hyphæ for the formation of zygospores.

A number of healthy hairs, removed from the heads of the children furnishing the fungus for my experiments, have been subjected to the same methods of cultivation, and have uniformly yielded a negative result.

Having thus followed the history of this fungus throughout its several stages, it will be proper, at this time, to ascertain the degree of confidence to be placed in the method of cultivation employed.

It is sufficiently evident that, after the completion of the cell, no contamination by foreign germs is to be feared. In the preparation of the cell, however, such accidents are liable to happen, and the unwelcome intruders are not slow to reveal themselves. The most frequent foreign germs are those of bacteria, which, however, are but little disposed to invade acidulated fluids. Penicillium, also, not unfrequently appears, but can always be traced, either by its hyphæ penetrating under the cover-glass, or radiating from a centre either in the surrounding fluid or at some point of the hair-shaft, and affording, in its style of growth, a striking contrast to the multitudinous, simultaneous outburst of activity from the hosts of trichophyton spores, along the whole shaft. In not a single instance where germination took place in the medusa-like manner I have described and figured, was there observed any other form of fructification than the sporangial one. Occasionally, during the growth of the hyphæ, a segmentation would occur indicating but not completing a breaking-up into torula-like chains of spores, beginning at the free end, the so-called brood-cell formation. Grawitz (*Virchow's Archiv*,

August, 1877) describes as derived from his slide and mass culture of achorion Schönleinii, trichophyton tonsurans, and microsporon furfur, a similar segmentation, and has concluded that these several forms are derivatives of oidium lactis, whose aërial fructification occurs in a somewhat similar centripetal segmentation. His drawings suggest, however, the torula-like brood-cell segmentations of other kinds of moulds.

It is of especial significance that, in cells whose conditions offer no obstacle to the development of ordinary mould fungus, the uniform result was constantly obtained, where the spores filling the hair germinated. And it is also of significance that, where this style of germination did not occur, the spores remained for weeks absolutely unchanged. It must be remarked, however, that the "trichophyton" does not germinate in cell culture with anything like the readiness of penicillium, aspergillus, or of ordinary mucor, or that itself displays in slide culture, where hyphæ are freely thrown out, but generally come to naught, on account of the unlimited growth of strange spores and bacteria. Indeed, although I have been able to follow in this slide culture the hyphal growth as far as the formation of sporangia, it has only been through my previous experience with cell culture that I have been able to distinguish the true parasite from the several other forms of fungus visible.

What the agencies are that prevent the free development of this fungus in cell cultivations, I am quite unable to say, although it is probable that the restricted supply of air has some influence. But it is a fact that successful results have been obtained in a very small minority of my cells, the vastly larger number remaining absolutely quiescent. This difficulty with which germination takes place is the only serious drawback to the method employed, which is one easily practised, readily available, guaranteeing the purity of the cultivation after the sowing; and, with scrupulous observance of all precautions in the preparations of the cells, their entire purity can be secured in a surprisingly large proportion. With a proper observance of details, and a patient persistence in the face of many failures, I am confident that my own observations will find confirmation at the hands of other investigators.

There remains, finally, the task of assigning the fungus of tinea tonsurans to its appropriate systematic position.

As has already been remarked, I have been unable to determine positively whether the increased area occupied by the spore mass, after growth has begun, is the result of the swelling of the spores, or of a positive spore increase through budding as well. I am, however, strongly impelled to adopt the latter opinion. But it must be borne in mind that this torula-like mode of growth does not imply more than a form of resemblance, and by no means the ferment-producing powers of yeast, and may be observed in a number of fungi (De Bary, "Morph. und Physiolog. der Pilze," pp. 119 and 182). At all events, in the cultivation of "trichophyton," this process ceases very early, as soon, indeed, as the hyphæ begin to grow freely.

In a hair invaded by "trichophyton" examined just after removal from the scalp, there will almost always be observed a decided tendency toward the division of the mycelium or hyphæ into very short segments, which bear every evidence of ultimately forming spores. This process, which I have frequently seen indicated in cell cultivation, but which I have never observed carried to completion, finds a perfect analogy in one of the reproductive processes of one of the mucors, mucor mucedo. De Bary says ("Morph. und Physiolog. der Pilze," p. 179) that in old mycelium, or in such as has, through deficient nourishment, deprivation of air, or other untoward influences, the formation of spores interfered with, short cylindrical sections filled with homogeneous protoplasm are formed by the appearance of septa, and become spores of a cylindrical, oval, or globular form. Doubtless, under more favorable conditions, this brood-cell formation is carried to its completion, in the development of trichophyton tonsurans; but for the present I must restrict myself to the statement that, in cell cultivations, this tendency is shown to be pretty constantly present. (*See* Fig. 3.)

In assigning "trichophyton" to the mucors, it will first be necessary to indicate some points in which the growth of the former differs from that usually ascribed to the latter. It will be observed that "trichophyton" departs from that feat-

ure characteristic of the family mucor, a unicellular, unsegmented condition of the hyphæ and mycelium previous to the formation of sporangia. This rule admits of some modification, however, since septa may appear when the protoplasmic contents become impoverished shortly before the sporangia begin to form. It must be remembered, moreover, that the formation of brood-cells requires a process of segmentation incompatible with an unvarying unicellular presporangial condition of the fungus, and depends usually upon unnatural and perverted influences. In slide cultures of "trichophyton," the hyphæ branch and attain a considerable length without the formation of septa; but these usually appear some time previous to the sporangia. In the cell cultivations, the septa appear earlier, although, where the hyphæ proceed immediately to the formation of sporangia, the septa may be absent.

Another point to be considered is the departure of this fungus from the type of sporangium development of mucor in the arrangement of the columella, which should project into the cavity of the sporangium in a conical shape. This may also, however, be regarded as a character subject to the altering effects of special influences. It will be observed that, of the sporangia represented in the drawings (Figs. 2 and 3), the greater number present only the straight septa dividing the sporangia from the hyphæ, others seeming to have been arrested in their growth before reaching this stage. Brefeld ("Botanische Untersuchungen über Schimmelpilze," Heft 11, p. 20) says that "sporangia starved or injured in their development or attacked by parasites vary greatly in size, and gradually lose the typical characters of mucor. The columella loses it shape, becomes smaller, and finally is entirely absent." The spores, according to Brefeld, may also diminish from their normal size, measuring sometimes as little as .0033 millimetre. It would seem, therefore, that the normal characters of mucor may be considerably altered by various disturbing influences; and, with the knowledge thus gained, it seems to me that a fungus presenting the features displayed in my cell cultivations may without hesitation be referred to the mucors.

In conclusion, I desire to express my sense of the imper-

fections of this paper, and my regret that I have been able to bring but a limited mycological experience to its preparation. I feel confident, however, that my observations as described and figured are correct, and that they will be confirmed by other investigators, employing the same methods of research.

The granular markings of the protoplasm in the illustration are rather too coarse.

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